BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

US-PAT-NO: 5529914

DOCUMENT-IDENTIFIER: US 5529914 A

TITLE: Gels for encapsulation of biological materials

---------- KWIC ---------

PEG diacrylate macromers may be polymerized identically as the PEG
tetraacrylate macromer described in this example.

This method takes advantage of the hydrophilic nature of PEG monomers.
Alginate/PLL microspheres (2 mL), containing one or two human pancreatic islets
each, were mixed with PEG tetraacrylate macromer solution (PEG mol wt 18.5 kD,
23% solution in saline) in a 50 mL transparent centrifuge tube.
Triethanolamine (0.1M) and 0.5 mM ethyl eosin were mixed with macromer
solution. The excess of macromer solution was decanted, 20 mL of mineral oil
was added to the tube, and the reaction mixture was vortexed thoroughly for 5
minutes. Silicone oil will perform equally well in this synthesis but may have
poorer adjuvant characteristics if there is any carry-over. Any other
water-immiscible liquid may be used as the "oil" phase. Acceptable
triethanolamine concentrations range from about 1 mM to about 100mM.
Acceptable ethyl eosin concentrations range from about 0.01 mM to more than 10
mM.

This example makes use of the direct interfacial polymerization. Islets of
Langerhans isolated from a human pancreas were encapsulated in PEG
tetraacrylate macromer gels. 500 islets suspended in RPMI 1640 medium

containing 10% fetal bovine serum were pelleted by centrifuging at 100 g for 3
min. The pellet was resuspended in 1 mL of a 23% w/v solution of PEO 18.5 kD
diacrylate macromer in HEPES buffered saline. An ethyl eosin solution (5
.mu.L) in vinyl pyrrolidone (at a concentration of 0.5%) was added to this
solution along with 100 .mu.L of a 5M solution of triethanolamine in saline.
Mineral oil (20 mL) was then added to the tube which was vigorously agitated to
form a dispersion of droplets 200-500 .mu.m in size. This dispersion was then
exposed to an argon ion laser with a power of 250 mW, emitting at 514 nm, for
30 sec. The mineral oil was then separated by allowing the microspheres to
settle, and the resulting microspheres were washed twice with PBS, once with
hexane and finally thrice with media.

The process also works at lower temperatures. For cellular encapsulation, a
23% solution of PEO diacrylate was used with initiating and polymerization
conditions as used in the air atomization technique. Cell viability subsequent
to encapsulation was checked by trypan blue exclusion assay. Human foreskin
fibroblasts (HFF), Chinese hamster ovary cells (CHO-Kl), and a beta cell
insuloma line (RiN5F) were found to be viable (more than 95%) after
encapsulation. A wide range (>10%) of PEG diacrylate concentrations may be
used equally effectively, as may PEG tetraacrylate macromers.

Solutions of PEO diacrylates (23% w/v; 0.4 kD, 6 kD, 10 kD) and PEG
tetraacrylates (18.5 kD) were used. An initiator solution (10 .mu.L)
containing 30 mg/mL of 2,2-dimethoxy-2-phenyl acetophenone in
vinyl-2-pyrrolidone was used per mL of the macromer solution. The solution of
initiator containing macromer was placed in a

4.0.times.1.0.times.0.5 cm mold
and exposed to a long wave ultraviolet lamp (365 nm) for approximately 10
seconds to induce gelation. Samples were allowed to equilibrate in phosphate
buffered saline (pH 7.4) for 1 week before analysis 1 performed.

For the creep tests, eight samples approximately 0.2.times.0.4.times.2 cm were
loaded while submersed in saline solution. They were tested with a constant
unique predetermined load for one hour and a small recovery load for ten
minutes. Gels made from PEG diacrylates of 1 kD, 6 kD, and 10 kD, and PEG
tetraacrylates of 18.5 kD PEO molecular weight were used for this study. The
10 kD test wets terminated due to a limit error (the sample stretched beyond
the travel of the loading frame). The 1 kD sample was tested with a load of 10
g and a recovery load of 0.2 g. The 6 kD sample was tested at a load of 13 g
with a recovery load of 0.5 g. The 18.5 kD sample was tested at a load of 13 g
with a recovery load of 0.2 g. The choice of loads for these samples produced
classical creep curves with primary and secondary regions.

PEG diacrylate (10 kD) and PEG--tetraacrylate (18.5 kD) were cast in dogbone
shapes as described in Example 19. PEG-dacrylate or tetraacrylate (23% w/w) in
sterile HEPES buffered saline (HBS) (0.9% NaCl, 10--HEPES, pH 7.4) containing
900 ppm of 2,2-dimethoxy-2-phenoxyacetophenone as initiator, was poured into an
aluminum mold and irradiated with a LWUV lamp (Black ray) for 1 min. The
initial weights of these samples were found after oven-drying these gels to
constant weight. The samples were soxhlet-extracted with methylene chloride
for 36 hours in order to leach out any unreacted prepolymer from the gel matrix
(sol-leaching) prior to testing. The process of extraction was continued until

the dried gels gave constant weight.

Tensile stress strain test was conducted on both control (unimplanted) and explanted dogbones in a small horizontal Instron-like device. The device is an aluminum platform consisting of two clamps mounted flat on a wooden board between two parallel aluminum guide. The top clamp was stationary while the bottom clamp was movable. Both the frictional surfaces of the moving clamp and the platform were coated with aluminum backed Teflon (Cole-Parmer) to minimize frictional resistance. The moving clamp was fastened to a device capable of applying a gradually increasing load. The whole set up was placed horizontally under a dissecting microscope (Reichert) and the sample elongation was monitored using a video camera. The image from the camera was acquired by an image processor (Argus-10, Hamamatsu) and sent to a monitor. After breakage, a cross section of the break surface was cut and the area measured. The load at break was divided by this cross section to find the maximum tensile stress.
Table 8 lists the stress at fracture of PEG tetraacrylate (18.5 kD) hydrogels explanted at various time intervals. No significant change in tensile strength was evident with time. Thus, the gels appear mechanically stable to biodegradation in vivo within the maximum time frame of implant in mice.

Disc shaped PEG-tetraacrylate hydrogels (m.w. 18.5 kD) were implanted intraperitoneally in mice as mentioned above for a period of 1 week, 3 weeks, 6 weeks, or 8 weeks. Explanted gels were rinsed in HBS twice and treated with Pronase (Calbiochem) to remove cells and cell debris. The samples were then equilibrated in HBS to let free Ca.sup.++ diffuse out from the gel matrix. The gels were then oven-dried (Blue-M) to a constant weight and

transferred to
Aluminum oxide crucibles (COORS, high temperature resistant). They were
incinerated in a furnace at 700.degree. C. for at least 16 hours. Crucibles
were checked for total incineration, if any residual remnants or debris was
seen they were additionally incinerated for 12 hours. Subsequently, the
crucibles were filled with 2 mL of 0.5M HCl to dissolve Ca.sup.++ salt and
other minerals in the sample. This solution was filtered and analyzed with
atomic absorption spectroscopy (AA) for calcium content.

Calcification data on PEG-tetraacrylate (mol. wt. 18.5 kD) gel implants is
given in Table 9. No significant increase in calcification was observed up to
an 8 week period of implantation in mice.

A formulation of PEG tetraacrylate (10%, 18.5 kD), was used as adhesive for
stabilizing the sutureless apposition of the ends of transected sciatic nerves
in the rat. Rats were under pentobarbital anesthesia during sterile surgical
procedures. The sciatic nerve was exposed through a lateral approach by
deflecting the heads of the biceps femoralis at the mid-thigh level. The
sciatic nerve was mobilized for approximately 1 cm and transected with
iridectomy scissors approximately 3 mm proximal to the tibeal-peroneal
bifurcation. The gap between the ends of the severed nerves was 2-3 mm. The
wound was irrigated with saline and lightly swabbed to remove excess saline.
Sterile, unpolymerized PEG tetraacrylate solution was applied to the wound.
Using delicate forceps to hold the adventitia or perineurium, the nerve ends
were brought into apposition, the macromer solution containing
2,2-dimethoxy-2-phenylacetophenone as a photoinitiator and the wound was
exposed to long wavelength UV-light (365 nm) for about 10

sec to polymerize the
adhesive. The forceps were gently pulled away. Care was taken to prevent the
macromer solution from flowing between the two nerve stumps. Alternatively,
the nerve stump junction was shielded from illumination, e.g. with a metal
foil, to prevent gelation of the macromer solution between the stumps; the
remaining macromer solution was then simply washed away.

It is possible to initiate photopolymerization with a wide variety of dyes as
initiators and a number of electron donors as effective cocatalysts. Table 10
illustrates photopolymerization initiated by several other dyes which have
chromophores absorbing at widely different wavelengths. All gelations were
carried out using a 23% w/w solution of 18.5 kD PEG tetraacrylate in HEPES
buffered saline. These initiating systems compare favorably with conventional
thermal initiating systems, as can also be seen from Table 10.

5. The method of claim 4 wherein the PEG is PEG tetraacrylate which has a
molecular weight around 18,500 D.

38. The method of claim 37 wherein the PEG is PEG tetraacrylate which has a
molecular weight around 18,500 D.

US-PAT-NO: 5573934

DOCUMENT-IDENTIFIER: US 5573934 A

TITLE: Gels for encapsulation of biological materials

---------- KWIC ---------

Examples of A include PEG diacrylate, from a PEG diol; of B include PEG
triacrylate, formed from a PEG triol; of C include PEG-cyclodextrin
tetraacrylate, formed by grafting PEG to a cyclodextrin central ring, and
further acrylating; of D include PEG tetraacrylate, formed by grafting two PEG
diols to a bis epoxide and further acrylating; of E include hyaluronic acid
methacrylate, formed by acrylating many sites on a hyaluronic acid chain; of F
include PEG-hyaluronic acid-multiacrylate, formed by grafting PEG to hyaluronic
acid and further acrylating; of G include PEG-unsaturated diacid ester formed
by esterifying a PEG diol with an unsaturated diacid.

PEG diacrylate macromers can be polymerized identically as the PEG
tetraacrylate macromer described in this example.

This method takes advantage of the hydrophilic nature of PEG monomers. 2 ml of
alginate/PLL microspheres, containing one or two human pancreatic islets each,
were mixed with PEG tetraacrylate macromer solution (PEG mol wt 18.5 kD, 23%
solution in saline) in a 50 ml transparent centrifuge tube. Triethanolamine
(0.1M) and 0.5 mM ethyl eosin were mixed with macromer solution. The excess
macromer solution was decanted, 20 ml of mineral oil was added to the tube, and
the reaction mixture was vortexed thoroughly for 5 minutes. Silicone oil will

perform equally well in this synthesis but may have poorer adjuvant characteristics if there is any carry-over. Any other water-immiscible liquid may be used as the "oil" phase. Acceptable triethanolamine concentrations range from about 1 mM to about 100 mM. Acceptable ethyl eosin concentrations range from about 0.01 mM to more than 10 mM.

This example makes use of the direct interfacial polymerization. Islets of Langerhans isolated from a human pancreas were encapsulated in PEG tetraacrylate macromer gels. 500 islets suspended in RPMI 1640 medium containing 10% fetal bovine serum were pelleted by centrifuging at 100 g for 3 min. The pellet was resuspended in 1 ml of a 23% w/v solution of PEG 18.5 K tetraacrylate macromer in HEPES buffered saline. 5 .mu.l of an ethyl eosin solution in vinyl pyrrolidone* (at a concentration of 0.5%) was added to this solution along with 100 .mu.l of a 5M solution of triethanolamine in saline. 20 ml of a mineral oil was then added to the tube which was vigorously agitated to form a dispersion of droplets 200-500 .mu.m in size. This dispersion was then exposed to an argon ion laser with a power of 250 mW, emitting at 514 nm, for 30 sec. The mineral oil was then separated by allowing the microspheres to settle, and the resulting microspheres were washed twice with phosphate buffered saline (PBS), once with hexane and three times with media.

FIG. 4 shows islets of Langerhans encapsulated in a PEG-tetraacrylate gel. The viability of the Islets was verified by an acridine orange and propidium iodide staining method and also by dithizone staining. In order to test functional normalcy, a SGS test was performed on these islets. The response of the encapsulated islets was compared to that of free islets

maintained in culture
for the same time period. All islets were maintained in culture for a week
before the SGS was performed. The results are summarized in Table 2. It can
be seen that the encapsulated islets secreted significantly (p<0.05) higher
amounts of insulin than the free islets. The PEG-tetraacrylate gel
encapsulation process did not impair function of the islets and in fact helped
them maintain their function in culture better than if they had not been
encapsulated.

The process also works at lower temperatures. For cellular encapsulation, a
23% solution of PEG diacrylate was used with initiating and polymerization
conditions as used in the air atomization technique. Cell viability subsequent
to encapsulation was checked with the trypan blue exclusion assay. Human
foreskin fibroblasts (HFF), Chinese hamster ovary cells (CHO-K1), and a beta
cell insuloma line (RiN5F) were found to be viable (more than 95%) after
encapsulation. A wide range of PEG diacrylate concentrations greater than 10%
can be used equally effectively, as can PEG tetraacrylate macromers.

In order to verify survival of cells after the overcoat process, cells in
suspension without the alginate/PLL microcapsule were exposed to similar
polymerization conditions. 1 ml of lymphoblastic leukemia cells (RAJI)
(5.times.10.sup.5 cells) was centrifuged at 300 g for 3 min. 1 ml of a 0.04%
filter sterilized ethyl eosin solution was phosphate buffered saline (PBS) is
added and the pellet was resuspended. The cells were exposed to the dye for 1
min and washed twice with PBS and then pelleted. 10 .mu.l of triethanolamine
solution (0.1M) was added to the pellet and the tube was vortexed to resuspend

the cells. 0.5 ml of PEG 18.5 K tetraacrylate macromer was then mixed into this suspension and the resulting mixture exposed to an argon ion laser (514 nm, 50 mW) for 45 sec. The cells were then washed twice with 10 ml saline and once with media (RPMI 1640 with 10% FCS and 1% antibiotic, antimycotic). A thin membrane of PEG-tetraacrylate gel was observed forming around each individual cell.

The polymerization rate of the macromer will depend on the macromer concentration, the initiator concentration, and the functionality of the macromer, e.g., the difunctionality of a PEG diacrylate or the tetrafunctionality of a PEG tetraacrylate, as well as the degree of acrylation of the material.

10 .mu.l of an initiator solution containing 30 mg/ml of 2,2-dimethoxy-2-phenyl acetophenone in vinyl-2-pyrrolidone was added per ml to 23% w/v solutions of PEG diacrylates (0.4K, 6K, 10K) and PEG tetraacrylates (18.5K). The solution of initiator containing macromer was placed in a 4.0.times.1.0.times.0.5 cm mold and exposed to a long wave ultraviolet lamp (365 nm) for approximately 10 seconds to induce gelation. Samples were allowed to equilibrate in phosphate buffered saline (pH 7.4) for 1 week before analysis was performed.

For the creep tests, eight samples approximately 0.2.times.0.4.times.2 cm were loaded while submersed in saline solution. They were tested with a constant unique predetermined load for one hour and a small recovery load for ten minutes. Gels made from PEG diacrylates of 1 K, 6 K, and 10 K, and PEG tetraacrylates of 18.5 K PEG molecular weight were used for this study. The 10 K test was terminated due to a limit error (the sample

stretched beyond the travel of the loading frame). The 1 K sample was tested with a load of 10 g and a recovery load of 0.2 g. The 6 K sample was tested at a load of 13 g with a recovery load of 0.5 g. The 18.5 K sample was tested at a load of 13 g with a recovery load of 0.2 g. The choice of loads for these samples produced classical creep curves with primary and secondary regions. The traces for creep for the 1 K, 6 K; and 18.5 K samples appear in FIGS. 14A-C, respectively.

PEG diacrylate (10 K) and PEG tetraacrylate (mol, wt. 18.5 k) were cast in dogbone shapes as described in Example 15. 23% w/w PEG-diacrylate or tetraacrylate in sterile HEPES buffered saline (HBS) (0.9% NaCl, 10 mM HEPES, pH 7.4), containing 900 ppm of 2,2-dimethoxy-2-phenoxyacetophenone as initiator, was poured into an aluminum mold and irradiated with a LWUV lamp (Black ray) for 1 min. The initial weights of these samples were found after oven-drying these gels to constant weight. The samples were Soxhlet-extracted with methylene chloride for 36 hours in order to leach out any unreacted prepolymer from the gel matrix (solleaching), prior to testing. The process of extraction was continued until the dried gels gave constant weight.

Tensile stress strain test was conducted on both control (unimplanted) and explanted dogbones in a small horizontal Instron-like device. The device is an aluminum platform consisting of two clamps mounted flat on a wooden board between two parallel aluminum guide. The top clamp is stationary while the bottom clamp is movable. Both the frictional surfaces of the moving clamp and the platform are coated with aluminum backed Teflon (Cole-Parmer) to minimize frictional resistance. The moving clamp is fastened to a

device capable of
applying a gradually increasing load. The whole set-up is placed horizontally
under a dissecting microscope (Reichert) and the sample elongation is monitored
using a video camera. The image from the camera is acquired by an image
processor (Argus-10, Hamamatsu) and sent to a monitor. After breakage, a cross
section of the break surface is cut and the area measured. The load at break
is divided by this cross section to find the maximum tensile stress. Table 8
lists the stress at fracture of PEG tetraacrylate (18.5 K) hydrogels explanted
at various time intervals. No significant change in tensile strength is
evident with time. Thus, the gels appear mechanically stable to biodegradation
in vivo within the maximum time frame of implant in mice.

Disc shaped PEG-tetraacrylate hydrogels (mol. wt. 18.5 k) were implanted
intraperitoneally in mice as described above for a period of 1 week, 3 weeks, 6
weeks or 8 weeks. Explanted gels were rinsed in HBS twice and treated with
Pronase (Calbiochem) to remove cells and cell debris. The samples were then
equilibrated in HBS to let free Ca.sup.++ diffuse out from the gel matrix. The
gels were then oven-dried (Blue-M) to a constant weight and transferred to
Aluminum oxide crucibles (COORS, high temperature resistant). They were
incinerated in a furnace at 700.degree. C. for at least 16 hours. Crucibles
were checked for total incineration, if any residual remnants or debris was
seen they were additionally incinerated for 12 hours. Subsequently, the
crucibles were filled with 2 ml of 0.5M HCl to dissolve Ca.sup.++ salt and
other minerals in the sample. This solution was filtered and analyzed with
atomic absorption spectroscopy (AA) for calcium content.

Calcification data on PEG-tetraacrylate (mol. wt. 18.5 K)

gel implants is given in Table 9. No significant increase in calcification was observed up to an 8 week period of implantation in mice.

A formulation of PEG tetraacrylate (10%, 18.5 K), was used as adhesive for stabilizing the sutureless apposition of the ends of transected sciatic nerves in the rat. Rats were under pentobarbital anesthesia during sterile surgical procedures. The sciatic nerve was exposed through a lateral approach by deflecting the heads of the biceps femoralis at the mid-thigh level. The sciatic nerve was mobilized for approximately 1 cm and transected with iridectomy scissors approximately 3 mm proximal to the tibial-peroneal bifurcation. The gap between the ends of the severed nerves was 2-3 mm. The wound was irrigated with saline and lightly swabbed to remove excess saline. Sterile, unpolymerized PEG tetraacrylate solution was applied to the wound. Using delicate forceps to hold the adventitia or perineurium, the nerve ends were brought into apposition, the macromer solution containing 2,2-dimethoxy-2-phenoxyacetophenone as a photoinitiator applied to the nerve ends and the wound was exposed to long wavelength UV-light (365 nm) for about 10 sec to polymerize the adhesive. The forceps were gently pulled away. Care was taken to prevent the macromer solution from flowing between the two nerve stumps. Alternatively, the nerve stump junction was shielded from illumination, e.g., with a metal foil, to prevent gelation of the macromer solution between the stumps; the remaining macromer solution was then simply washed away.

TABLE 9

Calcification data on PEG-tetraacrylate (mol. wt. 18.5K) gel implants TIME

CALCIFICATION (mean .+-. error*)	(Days)	(mg Calcium/g of Dry gel wt.)
7	2.33 .+-. 0.20	21
0.88 .+-. 0.009	42	
1.08 .+-. 0.30	56	1.17 .+-. 0.26

*Error based on 90% confidence limits.